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(54) Title: INACTIVATION OF PATHOGENS USING HYDROXYMETHYLAMINES (57) Abstract <p>A method of inactivating viruses that may be present in a whole blood or blood product intended for administration to an individual is disclosed. The whole blood or blood product sample is treated with an effective quantity of a hydroxymethylamine of formula (I). An exemplary hydroxymethylamine is N-hydroxymethylglycine and salts thereof.</p> <div style="text-align: right; margin-top: 20px;"> $\begin{array}{c} R \\ \backslash \\ N-CH_2-OH \\ / \\ R^1 \end{array} \quad (I)$ </div>		

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INACTIVATION OF PATHOGENS USING HYDROXYMETHYLAMINESFIELD OF THE INVENTION

This invention relates to methods for inactivation of pathogens.

5 BACKGROUND OF THE INVENTION

Spread of infectious disease resulting from transfusion of contaminated blood, administration of contaminated blood products or handling or usage of objects that have come into contact with contaminated
10 blood and/or blood products has been well documented and is recognized as a major public health concern. Most notably, transmission of viral hepatitis and/or Acquired Immune Deficiency Syndrome (AIDS) through contaminated blood and blood products has received
15 widespread attention. However, viral hepatitis and AIDS are only two of the many diseases that can be spread through use of contaminated blood and blood products. Lesser known pathogens, such as T-cell lymphotropic viruses (Types I and II),
20 cytomegalovirus, Epstein-Barr virus, the parvoviruses and Plasmodium (malaria-causing) protozoa, may also be spread through contaminated blood and blood products. In addition, still other microorganisms that have not yet even been identified or recognized
25 as being pathogenic may be transmitted through contaminated blood and blood products and, therefore, similarly pose a serious public health risk. The HIV virus is illustrative of a pathogen that, until recently, was not even recognized. Today, there are
30 over 10 million people worldwide who have contracted AIDS, many of these people having contracted the disease through use of infected blood or blood products; however, less than two decades ago, AIDS

was not even a recognized disease. Thus, it is clear that there is a great need for a method for effectively inactivating pathogens in blood and blood products.

5 In response to this need a number of techniques have been devised for inactivating pathogens, particularly infectious viral agents, in blood and/or blood products. A review of many of these techniques is presented in Suomela, "Inactivation of Viruses-in
10 Blood and Plasma Products," *Transfusion Medicine Reviews*, Vol. VII, No. 1, pp. 42-57 (January 1993), which is incorporated herein by reference.

 One such technique which has been used to inactivate viruses in blood and/or blood products is
15 pasteurization. [See Burnouf-Radosevich et al., "A Pasteurized Therapeutic Plasma, " Infusionstherapie, 19:91-94 (1992)] The pasteurization of blood and/or blood products is most often effected by heating them in the liquid state for 10 hours at 60°C. A small
20 amount of protein stabilizer, such as caprylate or tryptophanate, is often added to the preparation. After pasteurization has been completed, the stabilizer typically must be removed from the preparation prior to its clinical use. As is the
25 case with many of the existing viral inactivation techniques discussed herein, pasteurization is more effective in inactivating enveloped viruses (i.e., viruses having a lipid envelope surrounding the viral capsid) than in inactivating non-enveloped viruses
30 (i.e., viruses which lack a lipid envelope surrounding the viral capsid).

Another technique which has been used to inactivate viruses in blood and/or blood products is the solvent/detergent (S/D) method. [See, for example, Hellstern et al., "Manufacture and in vitro
5 Characterization of a Solvent/Detergent-Treated Human Plasma, " Vox Sang. 63:178-185 (1992); Horowitz et al., "Solvent/Detergent-Treated Plasma: A Virus-Inactivated Substitute for Fresh Frozen Plasma," Blood, 79:826-831 (1992); and Piquet et al., "Virus
10 Inactivation of Fresh Frozen Plasma by a Solvent Detergent Procedure: Biological Results, Vox Sang. 63:251-256 (1992).] The S/D method, which is limited to use in inactivating enveloped viruses, involves treating a blood preparation with an organic mixture
15 which disrupts the lipid envelope of enveloped viruses. The disruption of the lipid envelope leads either to complete structural disruption of the virus or to destruction of the cell receptor recognition site on the virus. In either case, the virus is
20 rendered noninfectious. The solvent used in the S/D method is most often tri-(n-butyl)phosphate (TNBP), and the detergent is either Tween 80°, Triton X-100° or sodium deoxycholate. Temperature and time influence the efficacy of the S/D method, typical
25 temperatures being in the range of 24° C to 37°C, and the typical duration of treatment being at least 6 hours.

Still another technique which has been used to inactivate viruses in blood and/or blood products is
30 photochemical inactivation. [See Mohr et al., "Virus Inactivated Single-Donor Fresh Plasma Preparations," Infusiontherapie, 19:79-83 (1993); Wagner et al., "Differential sensitivities of viruses in red cell suspensions to methylene blue photosensitization,"

Transfusion, 34(6):521-526 (1994); Wagner et al., "Red cell alterations associated with virucidal methylene blue phototreatment," Transfusion, 33:30-36 (1993); Mohr et al., "No evidence for neoantigens in human plasma after photochemical virus inactivation," Ann. Hematol., 65:224-228 (1992); Lambrecht et al., "Photoinactivation of Viruses in Human Fresh Plasma by Phenothiazine Dyes in Combination with Visible Light," Vox Sang., 60:207-213 (1991), Goodrich et al., "Selective inactivation of viruses in the presence of human platelets: UV sensitization with psoralen derivatives," Proc. Nat. Acad. Sci. USA, 91:5552-5556 (1994); Virus Inactivation in Plasma Products, J.-J Morgenthaler, ed. Karger, NY (1989); and BioWorld Today, Vol. 4, No. 229, pages 1 and 4 (November 24, 1993).] The photochemical inactivation of a blood preparation typically involves treating the blood preparation with a photoactivatable chemical and then irradiating the preparation with light of a sufficient wavelength to activate the photoactivatable chemical. Examples of photoactivatable chemicals used in the photochemical inactivation of viruses present in blood preparations include psoralens, hypericin, methylene blue and toluidine blue. It is believed that psoralens, which have an affinity for nucleic acids, inactivate viruses by intercalating between viral nucleic acid base pairs and, in the presence of UVA light, forming a covalent bond with the viral nucleic acid, thereby preventing its transcription and/or replication. The manner in which hypericin, methylene blue and toluidine blue inactivate viruses is not as well-defined as that for psoralens. However, it is believed that these chemicals, when photoactivated, generate the highly reactive entity, singlet oxygen,

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which then attacks the cellular structure (e.g. viral envelope) of the virus.

Whereas photochemical inactivation has been largely successful in inactivating enveloped viruses, it has been largely unsuccessful in inactivating non-enveloped viruses. The failure of photochemical inactivation to inactivate non-enveloped viruses is significant since Poliovirus, Adenovirus, Hepatitis A and Parvovirus (Parvo B19) are among those non-enveloped viruses that are pathogenic to humans.

It should be noted that photochemical inactivation of the type described above has been most successful when applied to inactivating viruses in blood preparations lacking red blood cells (e.g., plasma). This is because blood preparations that include red blood cells typically absorb light at the same wavelengths used to photoactivate the chemicals.

Viral inactivation agents are substances that render viruses incapable of replication and proliferation. From the literature discussed above, one may conclude that viral inactivating compounds have been identified which are specifically toxic to blood borne viruses such that cells and proteins are not adversely affected. Still it is important to limit exposure of biological samples to viral inactivation agents, such as, for example, psoralens, hypericin, methylene blue, toluidine blue or a combination of tri-(n-butyl) phosphate and a detergent such as Tween 80, Triton X-100 or sodium deoxycholate to the minimum extent necessary to reduce potentially significant interactions that could lead to undesirable side effects.

U.S. Patent 4,337,269 (Berke et al.) disclosed a biocidal composition containing a compound, hydroxymethylaminoacetate (also referred to herein as hydroxymethylglycinate), which is produced by the
5 reaction of glycine or a salt of glycine with formaldehyde. In the aforementioned patent, hydroxymethylglycinate is said to be effective at inhibiting the growth of bacteria, yeasts and molds in a variety of substances susceptible to microbial
10 contamination, such as cosmetics, foodstuffs, pharmaceuticals, paints, cutting oils or fluids, agricultural products, oil drilling fluids, paper industry, embalming solutions, cold sterilization medical and dental equipment, cooling towers, fabric
15 impregnation, latexes, swimming pools, inks, household disinfectants, waxes and polishes, toilet bowl cleaners, bathroom cleaners, laundry detergents, soaps, wood preservatives, hospital and medical antiseptics and adhesives.

20 Sodium hydroxymethylglycinate is the active ingredient in the preservative SUTTOCIDETM, which is commercially available from Sutton Laboratories, Chatham, New Jersey. In certain promotional literature published by Sutton Laboratories,
25 SUTTOCIDETM is said to be active against Gram-negative and Gram-positive bacteria, yeast and mold and is suggested for use as a preservative in shampoos, hair conditioners and facial treatments.

U.S. Patent 4,980,176 (Berke et al.) disclosed a
30 composition containing one or more 3-isothiazolones and a compound which is a member selected from the group consisting of hydroxymethyl-aminoacetic acid, its salts and lower alkyl esters. The aforementioned

composition is described in the patent as being effective against bacteria, yeasts and molds. Suggested applications in the patent for the above-described composition include use as a preservative
5 in cosmetics, toiletries and household cleaning products, use as a biocide for synthetic latexes, emulsion paints and other coatings, adhesives, polishes, carpet backing compositions, surfactants, metalworking fluids, industrial and domestic water
10 treatment including cooling tower systems and swimming pools, adhesive mats, drilling mud formulations, painting pastes, spin finish emulsions, polymer dispersions and fuels and as a slimicide for slime control in the manufacture of paper from wood
15 pulp.

It is to be noted that nowhere in the foregoing patents or publications is it taught that the biocidal activity of hydroxymethylaminoacetic acid, its salts and/or lower alkyl esters can be extended
20 beyond bacteria, yeasts and molds to include viruses. Moreover, because most biocidal agents that are effective against bacteria, yeasts and molds are not effective against viruses, the foregoing patents and publications do not provide any reasonable basis for
25 one of ordinary skill in the art to expect that hydroxymethylaminoacetic acid and/or its derivatives would be effective in inactivating viruses.

It is also to be noted that nowhere in the foregoing patents or publications is it taught that
30 hydroxymethylaminoacetic acid and/or its derivatives can be used in blood and/or blood products. Moreover, because most biocidal agents that are effective against bacteria, yeast and/or molds cannot

be used to inactivate such pathogens in blood an/or blood products without adversely affecting the suitability of the treated blood and/or blood product for subsequent administration to a patient (due to
5 their toxicity and/or their reactivity with plasma proteins and certain other blood constituents), the foregoing patents and publications do not provide any reasonable basis for one of ordinary skill in the art to expect that hydroxymethylaminoacetic acid and/or
10 its derivatives could be used in blood and/or blood products without rendering the treated blood and/or blood product unsuitable for subsequent administration to a patient in need thereof.

Also of interest is Japanese Published
15 Application No. 62-195304, which disclosed that paraform (84%, 25g) was added to 98% diethanolamine (332g) at 40 degrees, and then stirred at 50-60 degrees for 1 hour to yield hydroxymethyldi-ethanolamine. It is also disclosed that
20 hydroxymethyl-diethanolamine, at 300-500ppm, controlled *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Proteus vulgaris* and *P. mirabilis* on nutrient agar plates. It is to be noted, however, that nowhere in the
25 foregoing Japanese application is it taught or suggested that hydroxymethyldiethanolamine has virucidal activity or that hydroxymethyldiethanolamine could be used in blood and/or blood products without adversely affecting the suitability of the
30 treated blood and/or blood product for subsequent administration to a patient in need thereof.

Therefore, a need exists for a method for treating blood which inactivates viruses without

adversely affecting the suitability of the treated blood and/or blood product for subsequent administration to an individual in need thereof.

SUMMARY OF THE INVENTION

5 The present invention is a method of inactivating a virus in a biological fluid, such as blood. In the method, the biological fluid is contacted with a hydroxymethylamine (HMA) in sufficient quantity to inactivate the virus (i.e., an effective amount). The biological fluid can be of
10 any type including, but not limited to, whole blood and a wide variety of blood components, including, but not limited to, red blood cells, red blood cell concentrate, platelets, platelet concentrate,
15 platelet rich plasma, platelet poor plasma, source plasma (plasmaphoresis plasma), fresh frozen plasma, plasma proteins (e.g., clotting factors VIII, X, etc.), and other body fluids, such as lymph, cerebrospinal fluid, semen, saliva, etc. While
20 targeted at the inactivation of viruses, the method is effective against other microorganisms as well. These microorganisms can be pathogenic or nonpathogenic and include bacteria, yeasts, molds and protozoa.

25 In one embodiment, this invention provides a method for inactivating a microorganism contained in a biological fluid. The method comprises the step of contacting the microorganism with an effective amount of a hydroxymethylamine (HMA). Suitable hydroxy-
30 methylamines include compounds of Formula (I)

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5 wherein:

R is chosen from the group consisting of hydrogen, alkyl, aryl, substituted alkyl, substituted aryl; and R¹ is chosen from the group consisting of acid-,
 10 amide-, hydroxy- or mercapto-functional alkyl; acid-, amide-, or hydroxy-functional aryl; acid-, amide-, or hydroxy-functional substituted alkyl; and acid-, amide-, or hydroxy-functional substituted aryl;
 or R and R¹ may be joined together to form an acid,
 15 amide or hydroxy-functional heterocyclic structure.

Preferred HMAs are those in which the functional group is an amide or an acid selected from the group consisting of carboxylate, phosphate, phosphonate, sulfate and sulfonate. Carboxylic acids are
 20 particularly preferred.

Preferred individual hydroxymethylamines having the amide functionality include hydroxymethylglycinamide, hydroxymethylpenicillinamide, hydroxymethylleucinamide, hydroxymethylacrylamide and hydroxymethylnicotinamide. Preferred hydroxymethylamines having the acid functionality include hydroxymethylglycine, hydroxymethylphosphonomethylglycine, hydroxymethyl-p-aminohippuric acid, hydroxymethylpropargylglycine, hydroxymethyl-o-phosphothreonine, hydroxymethylaminoadipic acid, hydroxymethyl-o-phosphoserine, hydroxymethylaminoethylphosphonic acid, hydroxymethylleucine, hydroxymethyl-β-alanine, hydroxymethylcysteine, hydroxymethylfolic acid, hydroxymethylamino-

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phosphonobutyric acid, hydroxymethylphenylalanine,
hydroxymethylaminophenylacetic acid, hydroxymethyl-o-
phosphorylethanolamine, hydroxymethylalanine,
hydroxymethylserine, hydroxymethylvaline,
5 hydroxymethylmethionine, hydroxymethylglutamic acid,
hydroxymethylaspartic acid, hydroxymethyllysine,
hydroxymethylproline, hydroxymethylmercaptopropionyl-
glycine, hydroxymethylaminoethyl hydrogen sulfate,
hydroxymethylpenicillamine, hydroxymethylornithine,
10 and hydroxymethylcysteine. Preferred hydroxymethyl-
amines having neither an acid nor an amide
functionality include hydroxymethylmercaptoethyl-
amine, hydroxymethylaminoethanol, hydroxymethyl-
aminopropanol and hydroxymethyldiethanolamine.

15 A particularly preferred HMA is hydroxymethyl-
glycine or a salt thereof.

According to the method of the invention, the
hydroxymethylamine and biological fluid are
preferably combined to produce a final concentration
20 of hydroxymethylamine of approximately 0.05 % - 3.0 %
by weight; the contact time is from 0.5 hours to 4
hours, and the temperature is maintained between
about 4° C and about 30° C.

In another aspect, the invention relates to a
25 method of processing a biological fluid intended for
administration to an individual in need thereof. The
method comprises the steps of: (a) treating the
biological fluid with an effective amount of a
pathogen-inactivating hydroxymethylamine, thereby
30 producing a treated biological fluid; and (b) then
removing free hydroxymethylamine from the treated
biological fluid.

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In another aspect the invention relates to a method of treating an individual in need of a biological fluid. The method comprises the steps of:
(a) treating the biological fluid with an effective
5 amount of a pathogen-inactivating hydroxymethylamine, thereby producing a treated biological fluid; and (b) administering the treated biological fluid to the individual in need thereof.

In another aspect, the invention relates to a
10 method of treating a biological fluid. The method comprises combining an effective amount of a virus-inactivating hydroxymethylamine with the biological fluid, whereby at least about a 10-fold reduction in plaque forming units of virus is realized.
15 Subsequently, the virus-inactivating compound can be removed from the biological fluid prior to its administration to an individual. If the biological fluid is blood, the treated blood can be returned to the individual from whom it was obtained.
20 Alternatively, the treated blood can be stored and administered later in time to the same individual or another individual in need thereof.

The method of the invention can also be used to inactivate pathogens present in bodily fluids other
25 than blood, and to disinfect medical instruments and analytical equipment that have come into contact with potentially contaminated blood. Similarly, the method of the invention can also be used to disinfect blood samples that are not intended for subsequent
30 administration to an individual, but rather, are intended for subsequent chemical analysis. Other possible applications of the invention are apparent to those skilled in the art.

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Additional objects, as well as features and advantages, of the present invention will be set forth in part in the detailed description which follows, and in part will be obvious from the

5 detailed description or may be learned by practice of the invention. Various embodiments of the inventions will be described in sufficient detail to enable those skilled in the art to practice the invention, and it is to be understood that other embodiments may

10 be utilized and that changes may be made without departing from the scope of the invention. The following detailed description is, therefore, not to be taken in a limiting sense, and the scope of the present invention is best defined by the appended

15 claims.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the method of use of HMA's as pathogen-inactivating agents, particularly viral-inactivating agents, in biological

20 samples, such as blood or blood products.

Use of HMA for treatment of pathogens

The present invention is based, in part, on the unexpected discovery that a HMA can be used to inactivate viruses in biological samples. The present

25 invention is also based, in part, on the unexpected discovery that hydroxymethylamines such as those of Formula (I), can be used to inactivate viruses in blood and/or blood products, without rendering the treated blood and/or blood products unsuitable for

30 subsequent administration to an individual.

Suitable pathogen-inactivating (e.g., virus-inactivating) HMA's for use in the invention can be readily selected by those skilled in the art using art-recognized methods and the test methodology set forth in the accompanying Examples. For the purposes of the specification and claims, an HMA that reduces the number of plaque-forming units (PFU) in a biological fluid by a factor of at least 10 (i.e. one log reduction) is a pathogen-inactivating HMA. The ability to reduce the number of PFUs can be assessed using art-recognized methods, one example of which is described in Example 1. If an HMA reduces the number of PFUs by at least one log unit, as assessed by the method described in Example 1, it is a pathogen-inactivating HMA. The term "hydroxymethyl" when used herein in connection with a particular amino compound, designates that the compound has a -CH₂OH substituent on the amino group.

Suitable alkyl groups in compounds of Formula (I) include straight chain, branched chain, and cyclic alkyl groups, containing one to about 22 carbon atoms, more preferably one to about 12 carbon atoms. When the alkyl group is a cyclic alkyl, 3-, 4-, 5-, 6-, and 7- membered rings are preferred.

Suitable aryl groups in compounds of Formula (I) include hydrocarbon aryl groups containing a 6-membered aromatic ring, such as phenyl, fused bicyclic systems such as α - and β -naphthyl, histidine, indenyl, tetralinyl, and the like, and monocyclic and polycyclic heteroaryl groups containing a 5- or 6- membered heteroaromatic ring, e.g., pyridyl, pyrimidinyl, quinolinyl, furanyl,

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thienyl, isothiazolyl, isoxazolyl, imidazolyl, 1H-pyrrolyl, indolyl, purinyl, and the like.

Suitable substituents in a substituted alkyl or substituted aryl group include halogen (e.g., fluoro, chloro, bromo), hydroxy, alkoxy (e.g., alkoxy containing one to 8 carbon atoms), alkylthio (e.g., alkylthio wherein the alkyl group contains one to 8 carbon atoms), lower alkyl (i.e., alkyl containing one to four carbon atoms), cycloalkyl (e.g., cyclopropyl, cyclopentyl, cyclohexyl), phenyl, benzyl, benzo, mercapto, or combinations thereof.

R and R₁ of Formula (I) can together form an N-heterocyclic structure (i.e., a cyclic structure wherein the hydroxymethylated nitrogen is an atom in the cyclic structure), such as a proline, pyrrolidine, piperidine, 2-pyrroline, indoline, aziridine, azetidine, and the like.

In a preferred embodiment, the HMA contains a carboxylic acid portion as part of at least one of the substituted alkyl or substituted aryl groups, so that the HMA falls within the broad class of N-hydroxymethylated aminoacids. The aminoacid is not restricted to naturally occurring α -aminoacids, although they provide particularly convenient starting materials, and their residues are optimally biocompatible.

When the functional group is acidic, salts of the HMA, such as metal salts (e.g. sodium and potassium salts), and ammonium salts may be used.

Preferred HMAs include hydroxymethylglycine, hydroxymethylphosphonomethylglycine, hydroxymethyl-p-aminohippurate, hydroxymethylpropargylglycine, hydroxymethyl-o-phosphothreonine, hydroxymethyl-
5 aminoadipate, hydroxymethyl-o-phosphoserine, hydroxymethylamino-ethylphosphonic acid, hydroxymethylleucine, hydroxymethyl- β -alanine, hydroxymethylcysteine, hydroxymethylfolate, hydroxymethylaminophosphonobutyric acid, and hydroxymethylphenylalanine, and their corresponding salts.
10 Hydroxymethylglycine, hydroxymethylfolate, hydroxymethylaminophosphonobutyric acid, hydroxymethylpropargylglycine and hydroxymethyl-o-phosphothreonine are particularly preferred.

15 Other suitable HMAs include hydroxymethylamino-phenylacetic acid, hydroxymethyl-o-phosphorylethanolamine, hydroxymethylalanine, hydroxymethylserine, hydroxymethylvaline, hydroxymethylmethionine, hydroxymethylglutamate, hydroxymethylaspartate,
20 hydroxymethyllysine, hydroxymethylproline, hydroxymethylmercaptopropionylglycine, hydroxymethylmercapto-ethylamine, hydroxymethylaminoethyl hydrogen sulfate, hydroxymethylamino-ethanol, hydroxymethylpenicillamine, hydroxymethylhydantion, hydroxymethyl-
25 ornithine, hydroxymethylcysteine, hydroxymethylamino-propanol, hydroxymethyldiethanolamine, and their corresponding salts.

HMAs can be readily synthesized by those skilled in the art. For example, to synthesize a
30 hydroxymethyl-aminoacid, one equivalent each of the corresponding amino acid, formalin and sodium hydroxide are combined.

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One application of this invention is a method of inactivating a pathogen present in a biological sample, such as blood or a blood product intended for administration to an individual in need thereof, said method comprising the step of treating the biological sample, such as blood or a blood product with an effective amount of a pathogen-inactivating acid- or hydroxy-functional HMA.

In accordance with the teachings of the present invention, treatment of a biological fluid, such as blood and/or a blood product, comprises combining an appropriate quantity of a pathogen-inactivating HMA with the biological sample and then allowing the sample to incubate for an appropriate period of time at a suitable temperature. The final concentration of the HMA in the sample is preferably approximately 0.05%-3.0%, more preferably approximately 0.5%. The incubation period is sufficiently long to inactivate pathogen in the sample, commonly from about 0.5 hour to about 4 hours, conveniently about 1 hour, and the incubation temperature is about 18°C to about 37°C, more preferably about 20°C to about 30°C. In a specific embodiment, the treatment of a blood and/or blood product comprises combining sodium hydroxymethylglycinate with the sample to give a final sodium hydroxymethylglycinate concentration of 0.5%, then allowing the sample to incubate for approximately 60 minutes at a temperature of about 30°C.

The above-described method can be used to inactivate viruses, bacteria, molds, yeasts, protozoa and other pathogens in biological samples, such as whole blood and a wide variety of blood components,

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including, but not limited to, whole blood, red blood cell component, red blood cell concentrate, platelet component, platelet concentrate, platelet rich plasma, platelet poor plasma, source plasma, fresh
5 frozen plasma and plasma proteins. As mentioned above, one advantageous aspect of the present method is that it does not render a biological sample unsuitable for subsequent administration (e.g., transfusion) to an individual.

10 It is believed that HMA's inactivate viruses either by reacting with the protein coat or with the component nucleic acids.

The method of the invention can also be used to inactivate pathogens present in bodily fluids other
15 than blood, and to disinfect medical instruments and analytical equipment that have come into contact with potentially contaminated biological samples, such as blood. Similarly, the method of the invention can be used to disinfect blood samples that are not intended
20 for subsequent administration to an individual, but rather, are intended for subsequent chemical analysis. Other possible applications of the invention are apparent to those skilled in the art.

The following examples are illustrative only and
25 should in no way limit the scope of the present invention.

EXAMPLE 1

Five microliters of T4 (American Type Culture Collection No. 11303-B4) virus stock and 14 μ L of
30 blood plasma were added to each of four tubes. 1 μ L of a 50% stock solution of sodium

hydroxymethylglycinate (International Specialty Products, Bound Brook, New Jersey) was added to a first tube, 1 μ L of a 10 mg/mL stock solution of diazolidinyl urea (a bactericide) was added to a second tube, 1 μ L of a 10 mg/mL stock solution of imidazolidinyl urea (a bactericide) was added to a third tube, and 1 μ L of Phosphate Buffered Saline (PBS) was added to the fourth tube. The mixtures were incubated at room temperature for 1 hour before sampling, diluting, mixing with host cells in soft agar and pouring onto solid medium. After overnight incubation at 37°C, the plaques were counted. The results are summarized below in TABLE I.

TABLE I

Sample	Inhibitor	Concentration in Mixture	PFUs (Plaque Forming Units)
1	Hydroxymethylglycinate	2.5%	22x10 ⁴
2	Diazolidinyl urea	0.5 mg/mL	14x10 ⁶
3	Imidazolidinyl urea	0.5 mg/mL	42x10 ⁶
4	None	0	66x10 ⁶

The above results show that, whereas the bactericides diazolidinyl urea and imidiazolidinyl urea were ineffective at the above-indicated concentrations at inactivating T4 virus, hydroxymethylglycinate exhibited strong viricidal activity.

EXAMPLE 2

Five microliters of T4 virus stock were added to each of fourteen tubes. Fourteen microliters of blood plasma were added to each of seven of the tubes, and 14 μ L of whole blood were added to each of the remaining seven tubes. One microliter of an appropriate hydroxymethylglycinate stock solution was

added to each of six of the plasma-containing tubes and to each of six of the whole blood-containing tubes to give the below-listed concentrations. One microliter of PBS was added to each of the remaining
5 tubes. After incubation at room temperature for 1 hour, samples from each tube were taken, diluted, mixed with host cells, and overlaid onto solid medium. After overnight incubation at 37°C, plaques
10 were counted. The results are summarized below in TABLE II.

TABLE II

Sample	Hydroxymethylglycinate Concentration	Plasma or Whole Blood	PFU
1	2.5%	Plasma	21x10 ⁴
2	1.25%	Plasma	71x10 ⁴
15 3	0.625%	Plasma	70x10 ⁴
4	0.25%	Plasma	58x10 ⁵
5	0.125% —	Plasma	36x10 ⁶
6	0.063%	Plasma	18x10 ⁷
7	0%	Plasma	60x10 ⁸
20 8	2.5%	Whole Blood	29x10 ⁴
9	1.25%	Whole Blood	50x10 ⁴
10	0.625%	Whole Blood	76x10 ⁴
11	0.25%	Whole Blood	43x10 ⁵
12	0.125%	Whole Blood	74x10 ⁶
25 13	0.063%	Whole Blood	21x10 ⁸
14	0%	Whole Blood	67x10 ⁸

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As can be seen from the results above, the inactivation of T4 was dependent upon the concentration of hydroxymethylglycinate. A concentration of 0.625% hydroxymethylglycinate led to a 4 log decrease in T4 over a period of 1 hour at room temperature.

EXAMPLE 3

Two hundred twenty-five microliters of T4 stock were added to each of twelve tubes. 4.75 μ L of whole blood were added to three of the tubes, 4.75 μ L of plasma were added to another three of the tubes and 4.75 μ L of PBS were added to still another three of the tubes, the remaining three tubes serving as controls. 25 μ L of a 50% solution of hydroxymethylglycinate were added to each of the tubes, except for the controls. The various mixtures were incubated at room temperature, and at the times indicated below samples were taken, diluted, mixed with host cells and overlaid onto solid medium. After overnight incubation at 37°C, plaques were counted. The results are summarized below in TABLE III.

TABLE III

	Sample	Hydroxymethylglycinate Present	Incubation Time	PFU's
	Whole Blood	Yes	15 minutes	27×10^7
5	Plasma	Yes	15 minutes	9×10^7
	PBS	Yes	15 minutes	25×10^8
	Control	No	15 minutes	ND
	Whole Blood	Yes	30 minutes	9×10^7
10	Plasma	Yes	30 minutes	23×10^6
	PBS	Yes	30 minutes	40×10^5
	Control	No	30 minutes	ND
	Whole Blood	Yes	60 minutes	14×10^6
15	Plasma	Yes	60 minutes	25×10^5
	PBS	Yes	60 minutes	14×10^4
	Control	No	60 minutes	50×10^7

As can be seen from the results above, higher levels of hydroxymethylglycinate are required to inactivate T4 in blood or in plasma than in buffer solution. As can also be seen, the efficacy of hydroxymethylglycinate appears to increase as incubation time increases.

EXAMPLE 4

Thirty microliters of T4 stock and 465 μ L of blood plasma were added to each of ten tubes. 5 μ L of a 50% stock solution of hydroxymethylglycinate were added to each of eight of the ten tubes to give a final hydroxy-methylglycinate concentration of 0.5%. The mixtures were incubated at the temperatures indicated below. At the times indicated below, samples were taken from each tube, diluted, mixed with host cells and overlaid onto solid medium.

After overnight incubation, plaques were counted.
The results are summarized below in TABLE IV.

TABLE IV

5	Incubation Time	Hydroxymethylglycinate Present	Incubation Temperature	PFU's
	30 minutes	Yes	5°C	11x10 ⁷
	30 minutes	Yes	21°C	10x10 ⁶
	30 minutes	Yes	30°C	16x10 ⁴
	30 minutes	Yes	37°C	22x10 ³
10	30 minutes	No	37°C	ND
	60 minutes	Yes	5°C	12x10 ⁶
	60 minutes	Yes	21°C	23x10 ⁴
	60 minutes	Yes	30°C	< 10 ³
	60 minutes	Yes	37°C	< 10 ³
15	60 minutes	No	37°C	80x10 ⁷

As can be seen from the above results, the inactivation of T4 in blood plasma using 0.5% hydroxymethylglycinate is temperature dependent. Significantly greater viral toxicity was seen at 30°C than at room temperature, and at 5°C only a 1 log drop in viable virus was observed.

EXAMPLE 5

Thirty microliters of T4 stock and 465 µL of packed red blood cells were added to each of ten tubes. 5 µL of a 50% stock solution of hydroxymethylglycinate were added to each of eight of the ten tubes to give a final hydroxymethylglycinate concentration of 0.5%. The mixtures were incubated at the temperatures indicated below. At the times indicated below, samples were taken from each tube, diluted, mixed with host cells and overlaid onto solid medium. After overnight incubation at 37°C,

plaques were counted. The results are summarized below in TABLE V.

TABLE V

5	Incubation Time	Hydroxymethylglycinate Present	Incubation Temperature	PFU's
	30 minutes	Yes	5°C	15x10 ⁷
	30 minutes	Yes	21°C	20x10 ⁶
	30 minutes	Yes	30°C	11x10 ⁵
	30 minutes	Yes	37°C	21x10 ⁴
10	30 minutes	No	37°C	ND
	60 minutes	Yes	5°C	44x10 ⁶
	60 minutes	Yes	21°C	10x10 ⁵
	60 minutes	Yes	30°C	24x10 ⁴
	60 minutes	Yes	37°C	< 10 ³
15	60 minutes	No	37°C	11x10 ⁶

As can be seen from the above results, the inactivation of T4 in packed whole blood is also temperature dependent. At 37°C, over a 5 log drop in viral viability was observed over the time of the experiment. At 5°C, the decrease in viral viability was only 2 logs.

EXAMPLE 6

Thirty microliters of T4 stock and 440 µL of blood plasma were added to each of twelve tubes. 30 µL of a 50% stock solution of hydroxymethylglycinate were added to each of ten of the twelve tubes to give a final hydroxymethylglycinate concentration of 3%. The mixtures were incubated at the temperatures indicated below. At the times indicated below, samples were taken from each tube, diluted, mixed with host cells and overlaid onto solid medium. After overnight incubation at 37°C, plaques were

counted. The results are summarized below in Table VI.

TABLE VI

5	Incubation Time	Hydroxymethylglycinate Present	Incubation Temperature	PFU's
	30 minutes	Yes	0°C	29x10 ⁷
	30 minutes	Yes	5°C	72x10 ⁶
	30 minutes	Yes	21°C	69x10 ⁵
	30 minutes	Yes	30°C	< 10 ³
10	30 minutes	Yes	37°C	< 10 ³
	30 minutes	No	37°C	ND
	60 minutes	Yes	0°C	28x10 ⁷
	60 minutes	Yes	5°C	35x10 ⁶
	60 minutes	Yes	21°C	75x10 ³
15	60 minutes	Yes	30°C	< 10 ³
	60 minutes	Yes	37°C	< 10 ³
	60 minutes	No	37°C	117x10 ⁷

As can be seen by comparing the above results to those obtained in Examples 4 and 5, 3% hydroxymethylglycinate possesses greater viricidal activity than does 0.5% hydroxymethylglycinate. The above results also indicate that viral inactivation by 3% hydroxymethylglycinate is temperature dependent. For example, a greater than 5 log decrease in viable virus was observed when the incubation temperature was increased from 0°C to 30°C.

EXAMPLE 7

Fifty microliters of an overnight culture of *E. coli* and 465 μ L of blood plasma were added to each of ten tubes. 5 μ L of a 50% stock solution of hydroxymethylglycinate were added to each of eight of

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the ten tubes to give a final hydroxymethylglycinate concentration of 0.5%. The mixtures were incubated at the temperatures indicated below. At the times indicated below, samples were taken from each tube, diluted, and plated. After overnight incubation at 37°C, colonies were counted. The results are summarized below in TABLE VII.

TABLE VII

10	Incubation Time	Hydroxymethylglycinate Present	Incubation Temperature	Colonies
	30 minutes	Yes	5°C	7×10^7
	30 minutes	Yes	21°C	11×10^6
	30 minutes	Yes	30°C	15×10^5
	30 minutes	Yes	37°C	$< 10^3$
15	30 minutes	No	37°C	ND
	60 minutes	Yes	5°C	41×10^6
	60 minutes	Yes	21°C	7×10^3
	60 minutes	Yes	30°C	$< 10^3$
	60 minutes	Yes	37°C	$< 10^3$
20	60 minutes	No	37°C	35×10^7

The above results indicate that hydroxymethylglycinate inactivates bacteria, as well as viruses, in blood plasma. The above results also indicate that the inactivation is temperature dependent.

EXAMPLE 8

Four hundred sixty-five microliters of blood plasma and 30 μ L of T4 stock were added to each of eight tubes. 5 μ L of 50% stock solution of hydroxymethylglycinate, pH 9.0, were added to each of two of the eight tubes. A sample of the aforementioned hydroxymethylglycinate stock solution

was adjusted to pH 7.8 with solid sodium phosphate, and 5 μ L of the pH-adjusted hydroxymethylglycinate solution were added to another two of the eight tubes. 5 μ L of buffer, pH 9.0, were added to another two of the eight tubes, the remaining two tubes serving as controls. The mixtures were incubated at 30°C. At the times indicated below, samples were taken, diluted, mixed with host cells and overlaid onto solid medium. After overnight incubation at 37°C, plaques were counted. The results are summarized below in TABLE VIII.

TABLE VIII

	ADDITIVE	INCUBATION TIME	PFU'S
15	Hydroxymethylglycinate pH 7.8	30 minutes	29x10 ³
	Hydroxymethylglycinate pH 9.0	30 minutes	18x10 ⁴
	Buffer pH 9.0	30 minutes	22x10 ⁷
20	None —	30 minutes	ND
	Hydroxymethylglycinate pH 7.8	60 minutes	<10 ³
	Hydroxymethylglycinate pH 9.0	60 minutes	10x10 ³
25	Buffer pH 9.0	60 minutes	78x10 ⁷
	None	60 minutes	26x10 ⁷

EXAMPLE 9

Each of the hydroxymethyl derivatives listed below was synthesized along the lines described in U.S. Patent No. 4,337,269 by mixing 10 mmoles of the corresponding L-amino acid with 10 mmoles of 50% aqueous sodium hydroxide and 10 mmoles of 37% formaldehyde. After overnight incubation at room

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temperature, no free formaldehyde could be detected in any of the reaction mixtures.

465 μ L of blood plasma and 30 μ L of T4 virus were added to each of nine tubes. In addition, to each tube was added 5 μ L of one of the hydroxymethyl derivatives listed below, the remaining tube having no hydroxymethyl derivative and serving as a control. The mixtures were incubated at 30°C for 60 minutes. Samples were then taken, diluted, mixed with host cells and overlaid onto solid medium. After overnight incubation at 37°C, plaques were counted. The results are summarized below in TABLE IX.

TABLE IX

	INHIBITOR	PFU's
15	Hydroxymethylglycine	$<10^3$
	Hydroxymethylalanine	14×10^3
	Hydroxymethylaspartate	7×10^4
	Hydroxymethyllysine	28×10^4
	Hydroxymethylornithine	33×10^5
20	Hydroxymethylproline	36×10^4
	Hydroxymethylserine	20×10^3
	Hydroxymethylvaline	54×10^3
	None	6×10^7

EXAMPLE 10

25 10 μ L of T4 virus stock and 89 μ L of blood plasma were added to each of seven tubes. 1 μ L of an appropriate hydroxymethylvaline stock solution was added to each of three of the plasma-containing tubes and 1 μ L of an appropriate hydroxymethylaspartate

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stock solution was added to each of another three of the plasma-containing tubes to give the below-listed concentrations. 1 μ L of a buffer solution was added to the remaining tube as a control. After incubation at 30°C for 1 hour, samples from each tube were taken, diluted, mixed with host cells, and overlaid onto solid medium. After overnight incubation at 37°C, plaques were counted. The results are summarized below in TABLE X.

10

TABLE X

INHIBITOR	CONCENTRATION	PFU'S
Hydroxymethylvaline	0.25%	35x10 ⁵
Hydroxymethylaspartate	0.25%	12x10 ⁵
Hydroxymethylvaline	0.1%	42x10 ⁵
Hydroxymethylaspartate	0.1%	13x10 ⁶
Hydroxymethylvaline	0.05%	57x10 ⁶
Hydroxymethylaspartate	0.05%	20x10 ⁶
None	0%	29x10 ⁷

15

EXAMPLE 11

20

T4 virus stock and blood plasma were added to each of six tubes in the manner described above. Hydroxymethyl-o-phosphorylethanolamine was added to each of two tubes, hydroxymethyl-trp-gly-gly was added to each of another two tubes, and a buffer containing no inhibitor was added to each of the remaining two tubes. The mixtures were incubated at 30°C. At the times indicated below, samples were taken from each tube, diluted, and plated. After overnight incubation at 37°C, plaques were counted. The results are summarized below in TABLE XI.

30

TABLE XI

	Inhibitor	Incubation Period	PFU's
	Hydroxymethyl-o-phosphorylethanolamine	30 minutes	11x10 ⁴
5	Hydroxymethyl-trp-gly-gly	30 minutes	17x10 ⁹
	None	30 minutes	ND
	Hydroxymethyl-o-phosphorylethanolamine	60 minutes	40x10 ³
	Hydroxymethyl-trp-gly-gly	60 minutes	18x10 ⁹
10	None	60 minutes	25x10 ⁹

EXAMPLE 12

465 μ L of blood plasma, 30 μ L of T4 stock solution were added to each of ten tubes. 5 μ L of a 50% solution of hydroxymethylaminopropanol were added to each of two tubes. 5 μ L of a 50% solution of hydroxymethylpenicillinamine were added to each of another two tubes. 5 μ L of a 50% solution of hydroxymethylcysteine ethyl ester were added to each of another two tubes. 5 μ L of PBS were added to each of another two tubes. 5 μ L of a solution containing equimolar amounts of NaOH and formaldehyde incubated overnight at room temperature were added to each of the two remaining tubes. The mixtures were incubated at 30°C. At the times indicated below, samples were taken, diluted, mixed with host cells and overlaid onto solid medium. After overnight incubation at 37°C, plaques were counted. The results are summarized below in TABLE XII.

TABLE XII

	Inhibitor	Incubation Period	PFU's
30	Hydroxymethylaminopropanol	30 minutes	11x10 ⁶
	Hydroxymethylpenicillinamine	30 minutes	8x10 ⁶
	Hydroxymethylcysteine ethyl ester	30 minutes	6x10 ⁷

5

Inhibitor	Incubation Period	PFU's
None	30 minutes	ND
Alkalinized formaldehyde	30 minutes	ND
Hydroxymethylaminopropanol	60 minutes	5×10^6
Hydroxymethylpenicillinamine	60 minutes	24×10^5
Hydroxymethylcysteine ethyl ester	60 minutes	6×10^7
None	60 minutes	16×10^7
Alkalinized formaldehyde	60 minutes	14×10^6

EXAMPLE 13

10 465 μ L of blood plasma, 30 μ L of T4 stock
solution were added to each of eight tubes. 5 μ L of
a 50% solution of hydroxymethylcysteine were added to
each of two tubes. 5 μ L of a 50% solution of hydro-
xymethyl-aminophenyl acetic acid were added to each
15 of another two tubes. 5 μ L of a 50% solution of
hydroxymethylaminoethanol were added to each of
another two tubes. 5 μ L of a buffer solution serving
as a control were added to each of the two remaining
tubes. The mixtures were incubated at 30°C. At the
20 times indicated below, samples were taken, diluted,
mixed with host cells and overlaid onto solid medium.
After overnight incubation at 37°C, plaques were
counted. The results are summarized below in TABLE
XIII.

TABLE XIII

	INHIBITOR	INCUBATION PERIOD	PFU's
	Hydroxymethylcysteine	30 minutes	7×10^7
5	Hydroxymethyl-aminophenyl acetic acid	30 minutes	23×10^4
	Hydroxymethylamino-ethanol	30 minutes	12×10^6
	None	30 minutes	ND
10	Hydroxymethylcysteine	60 minutes	34×10^6
	Hydroxymethyl-aminophenyl acetic acid	60 minutes	23×10^3
	Hydroxymethylamino-ethanol	60 minutes	11×10^5
	None	60 minutes	29×10^7

15 EXAMPLE 14

465 μ L of blood plasma, 30 μ L of T4 stock solution were added to each of eight tubes. 5 μ L of a 50% solution of hydroxymethylmercaptopropionylglycine were added to each of two tubes. 5 μ L of a 50% solution of hydroxymethylmercaptoethylamine were added to each of another two tubes. 5 μ L of a 50% solution of hydroxymethylaminoethyl hydrogen sulfate were added to each of another two tubes. 5 μ L of a buffer solution serving as a control were added to each of the two remaining tubes. The mixtures were incubated at 30°C. At the times indicated below, samples were taken, diluted, mixed with host cells and overlaid onto solid medium. After overnight incubation at 37°C, plaques were counted. The results are summarized below in TABLE XIV.

TABLE XIV

	INHIBITOR	INCUBATION PERIOD	PFU's
5	Hydroxymethylmercapto propionylglycine	30 minutes	15x10 ⁶
	Hydroxymethyl- mercaptoethylamine	30 minutes	20x10 ⁶
	Hydroxymethylamino- ethyl hydrogen sulfate	30 minutes	23x10 ⁶
10	None	30 minutes	ND
	Hydroxymethylmercapto- propionylglycine	60 minutes	36x10 ⁵
	Hydroxymethyl- mercaptoethylamine	60 minutes	22x10 ⁵
15	Hydroxymethylamino- ethyl hydrogen sulfate	60 minutes	20x10 ⁵
	None	60 minutes	17x10 ⁷

EXAMPLE 15

The virucidal activities of hydroxymethyl-p-aminohippurate, hydroxymethylpropargyl-glycine, and hydroxymethyl-o-phosphothreonine, respectively, were tested in the manner described above. The results are summarized below in TABLE XV.

TABLE XV

	INHIBITOR	INCUBATION PERIOD	PFU's
25	Hydroxymethyl-p-amino- hippurate	30 minutes	<10 ³
	Hydroxymethylpropargyl- glycine	30 minutes	<10 ³
30	Hydroxymethyl-o- phospho-threonine	30 minutes	1x10 ³
	None	30 minutes	ND
	Hydroxymethyl-p-amino- hippurate	60 minutes	<10 ³
35	Hydroxymethylpropargyl- glycine	60 minutes	<10 ³
	Hydroxymethyl-o- phospho-threonine	60 minutes	<10 ³

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INHIBITOR	INCUBATION PERIOD	PFU's
None	60 minutes	4×10^7

EXAMPLE 16

The virucidal activities of hydroxymethyl-aminoadipate, hydroxymethyl-o-phosphoserine, and hydroxymethylaminoethylphosphonic acid, respectively, were tested in the manner described above. The results are summarized below in TABLE XVI.

TABLE XVI

	INHIBITOR	INCUBATION PERIOD	PFU's
10	Hydroxymethylamino-adipate	30 minutes	5×10^3
	Hydroxymethyl-o-phosphoserine	30 minutes	$<10^3$
15	Hydroxymethylamino-ethylphosphonic acid	30 minutes	2×10^3
	None	30 minutes	ND
	Hydroxymethylamino-adipate	60 minutes	10^3
20	Hydroxymethyl-o-phosphoserine	60 minutes	$<10^3$
	Hydroxymethylamino-ethylphosphonic acid	60 minutes	$<10^3$
	None	60 minutes	24×10^7

EXAMPLE 17

465 μ L of blood plasma and 30 μ L of T4 were added to each of six tubes. 5 μ L of hydroxymethylphosphonomethylglycine were added to each of two tubes, and 5 μ L of hydroxymethylmethyldantion were added to each of another two tubes. 5 μ L of buffer were added to each of the remaining two tubes. The

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mixtures were incubated at 30°C. At the times indicated below, samples were taken, diluted, mixed with host cells and overlaid onto solid medium. After overnight incubation at 37°C, plaques were counted. The results are summarized below in TABLE XVII.

TABLE XVII

	Inhibitor	Incubation Period	PFU's
10	Hydroxymethylphosphono-methylglycine	30 minutes	28x10 ⁴
	Hydroxymethylmethylhydantion	30 minutes	13x10 ⁶
	None	30 minutes	ND
	Hydroxymethylphosphono-methylglycine	60 minutes	< 10 ³
15	Hydroxymethylmethylhydantion	60 minutes	37x10 ⁵
	None	60 minutes	25x10 ⁶

EXAMPLE 18

The virucidal activities of hydroxymethylglycine-7-amido-4-methylcoumarin, hydroxymethyl-vinylglycinate, hydroxymethylfolic acid, hydroxymethyltaurine and hydroxymethyl-aminoethyl trimethyl ammonium chloride, respectively, were tested in the manner described above at the concentrations indicated below. The results are summarized below in TABLE XVIII.

TABLE XVIII

	Inhibitor	Inhibitor Concentration	PFU's-
5	Hydroxymethylglycine-7-amido-4-methylcoumarin	0.5%	12×10^5
	Hydroxymethylvinylglycinate	0.5%	$< 10^3$
	Hydroxymethylfolic acid	0.5%	$< 10^3$
	Hydroxymethyltaurine	0.5%	25×10^4
	Hydroxymethylaminoethyl trimethyl ammonium chloride	0.5%	40×10^6
10	Hydroxymethylglycine-7-amido-4-methylcoumarin	0.25%	21×10^5
	Hydroxymethylvinylglycinate	0.25%	88×10^3
	Hydroxymethylfolic acid	0.25%	$< 10^3$
	Hydroxymethyltaurine	0.25%	22×10^5
	Hydroxymethylaminoethyl trimethyl ammonium chloride	0.25%	56×10^6
15	Hydroxymethylglycine-7-amido-4-methylcoumarin	0.1%	19×10^5
	Hydroxymethylvinylglycinate	0.1%	67×10^4
	Hydroxymethylfolic acid	0.1%	10×10^6
	Hydroxymethyltaurine	0.1%	30×10^5
	Hydroxymethylaminoethyl trimethyl ammonium chloride	0.1%	38×10^6
20	Hydroxymethylglycine-7-amido-4-methylcoumarin	0.1%	19×10^5
	Hydroxymethylvinylglycinate	0.1%	67×10^4
	Hydroxymethylfolic acid	0.1%	10×10^6
	Hydroxymethyltaurine	0.1%	30×10^5
	Hydroxymethylaminoethyl trimethyl ammonium chloride	0.1%	38×10^6
	None	-	15×10^7

25 EXAMPLE 19

The virucidal activities of
 hydroxymethylglycinate, hydroxymethylaminoadipate,
 hydroxymethylaminoethylphosphonic acid, and
 hydroxymethyl-o-phosphoserine, respectively, were
 30 tested in the manner described above at the con-
 centrations indicated below. The results are
 summarized below in TABLE XIX.

TABLE XIX

	Inhibitor	Inhibitor Concentration	PFU's
	Hydroxymethylglycinate	0.25%	9×10^5
	Hydroxymethylaminoadipate	0.25%	14×10^6
5	Hydroxymethylaminoethylphosphonic acid	0.25%	22×10^5
	Hydroxymethyl-o-phosphoserine	0.25%	32×10^5
	Hydroxymethylglycinate	0.1%	16×10^5
	Hydroxymethylaminoadipate	0.1%	20×10^6
10	Hydroxymethylaminoethylphosphonic acid	0.1%	7×10^6
	Hydroxymethyl-o-phosphoserine	0.1%	14×10^6
	Hydroxymethylglycinate	0.05%	2×10^6
	Hydroxymethylaminoadipate	0.05%	7×10^7
15	Hydroxymethylaminoethylphosphonic acid	0.05%	31×10^6
	Hydroxymethyl-o-phosphoserine	0.05%	20×10^6
	None	-	5×10^7

EXAMPLE 20

20 The virucidal activities of hydroxymethylphosphonomethylglycinate and hydroxymethylglycinamide, respectively, were tested in the manner described above at the concentrations indicated below. The results are summarized below in TABLE XX.

TABLE XX

	Inhibitor	Inhibitor Concentration	PFU's
	Hydroxymethylphosphono-methylglycinate	0.5%	ND
5	Hydroxymethylglycinamide	0.5%	99x10 ⁴
	Hydroxymethylphosphono-methylglycinate	0.25%	49x10 ⁵
	Hydroxymethylglycinamide	0.25%	15x10 ⁶
10	Hydroxymethylphosphono-methylglycinate	0.1%	34x10 ⁶
	Hydroxymethylglycinamide	0.1%	72x10 ⁶
	Hydroxymethylphosphono-methylglycinate	0.05%	55x10 ⁶
	Hydroxymethylglycinamide	0.05%	ND
15	None	-	55x10 ⁷

EXAMPLE 21

The virucidal activities of hydroxymethylglycinate, hydroxymethylaminohippurate, hydroxymethylpropargylglycine and hydroxymethyl-O-phosphothreonine, respectively, were tested in the manner described above at the concentrations indicated below. The results are summarized below in TABLE XXI.

TABLE XXI

	Inhibitor	Inhibitor Concentration	PFU's
	Hydroxymethylglycinate	0.25%	15x10 ⁴
	Hydroxymethylaminohippurate	0.25%	54x10 ⁶
5	Hydroxymethylpropargylglycine	0.25%	27x10 ³
	Hydroxymethyl-O-phosphothreonine	0.25%	10x10 ³
	Hydroxymethylglycinate	0.1%	7x10 ⁶
	Hydroxymethylaminohippurate	0.1%	45x10 ⁶
	Hydroxymethylpropargylglycine	0.1%	41x10 ⁵
10	Hydroxymethyl-O-phosphothreonine	0.1%	36x10 ⁴
	Hydroxymethylglycinate	0.05%	43x10 ⁶
	Hydroxymethylaminohippurate	0.05%	44x10 ⁶
	Hydroxymethylpropargylglycine	0.05%	29x10 ⁶
	Hydroxymethyl-O-phosphothreonine	0.05%	15x10 ⁶
15	None	-	32x10 ⁷

EXAMPLE 22

The virucidal activities of hydroxymethylthreonine, hydroxymethylphosphothreonine, hydroxymethylserine and hydroxymethylphosphoserine, respectively, were tested in the manner described above at the concentrations indicated below. The results are summarized below in TABLE XXII.

TABLE XXII

	Inhibitor	Inhibitor Concentration	PFU's
	Hydroxymethylthreonine	0.25%	86x10 ⁴
	Hydroxymethylphosphothreonine	0.25%	75x10 ⁴
5	Hydroxymethylserine	0.25%	14x10 ⁶
	Hydroxymethylphosphoserine	0.25%	42x10 ⁵
	Hydroxymethylthreonine	0.1%	45x10 ⁶
	Hydroxymethylphosphothreonine	0.1%	30x10 ⁵
	Hydroxymethylserine	0.1%	22x10 ⁶
10	Hydroxymethylphosphoserine	0.1%	21x10 ⁶
	Hydroxymethylthreonine	0.05%	5x10 ⁶
	Hydroxymethylphosphothreonine	0.05%	43x10 ⁵
	Hydroxymethylserine	0.05%	17x10 ⁷
	Hydroxymethylphosphoserine	0.05%	51x10 ⁶
15	None	-	42x10 ⁷

EXAMPLE 23

The virucidal activities of hydroxymethyl-MTH-glycine, hydroxymethyl-1-amino-1-cyclopropane carboxylic acid, hydroxymethyl-d,l-2-aminophosphonopropionic acid, hydroxy-methyl-p-aminobenzoic acid, hydroxymethylamino-butyrolactone, hydroxymethyl-d,l-aminophosphonobutyric acid, hydroxymethylaminopyrazole carboxylic acid, hydroxymethylazetidine carboxylate and hydroxymethyldiaminobutyric acid, respectively, were tested in the manner described above at the concentrations indicated below. The results are summarized below in TABLE XXIII.

TABLE XXIII

	Inhibitor	Inhibitor Concentration	PFU's
5	Hydroxymethyl-MTH-glycine	0.5%	13×10^6
	Hydroxymethyl-1-amino-1-cyclopropane carboxylic acid	0.5%	17×10^3
	Hydroxymethyl-d,1-2-aminophosphonopropionic acid	0.5%	$< 10^3$
	Hydroxymethyl-p-aminobenzoic acid	0.5%	5×10^3
10	Hydroxymethylaminobutyrolactone	0.5%	30×10^3
	Hydroxymethyl-d,1-aminophosphonobutyric acid	0.5%	$< 10^3$
	Hydroxymethylaminopyrazole carboxylic acid	0.5%	45×10^4
15	Hydroxymethyl azetidine carboxylate	0.5%	7×10^3
	Hydroxymethyldiaminobutyric acid	0.5%	$< 10^3$
	Hydroxymethyl-MTH-glycine	0.25%	15×10^7
20	Hydroxymethyl-1-amino-1-cyclopropane carboxylic acid	0.25%	18×10^4
	Hydroxymethyl-d,1-2-aminophosphonopropionic acid	0.25%	11×10^4
	Hydroxymethyl-p-aminobenzoic acid	0.25%	30×10^4
25	Hydroxymethylaminobutyrolactone	0.25%	ND
	Hydroxymethyl-d,1-aminophosphonobutyric acid	0.25%	$< 10^3$
	Hydroxymethylaminopyrazole carboxylic acid	0.25%	21×10^4
30	Hydroxymethyl azetidine carboxylate	0.25%	16×10^4
	Hydroxymethyldiaminobutyric acid	0.25%	40×10^5
	Hydroxymethyl-MTH-glycine	0.1%	18×10^7
35	Hydroxymethylglutamate	0.25%	60×10^4
	Hydroxymethylmethionine	0.25%	68×10^5
	Hydroxymethylserine	0.25%	25×10^3
	Hydroxymethylglutamate	0.1%	12×10^6
	Hydroxymethylmethionine	0.1%	28×10^6
	Hydroxymethylserine	0.1%	27×10^5

Inhibitor	Inhibitor Concentration	PFU's
Hydroxymethylglutamate	0.05%	9×10^7
Hydroxymethylmethionine	0.05%	7×10^7
Hydroxymethylserine	0.05%	7×10^6
None	-	9×10^8

5 EXAMPLE 24

89 μL of blood plasma and 10 μL of T4 stock solution were added to each of ten tubes. 1 μL of an appropriate hydroxymethylglutamate stock solution was added to each of three tubes to give the
10 concentrations indicated below. 1 μL of an appropriate hydroxymethylmethionine stock solution was added to each of another three tubes to give the concentrations indicated below. 1 μL of an appropriate hydroxymethylserine stock solution was
15 added to each of another three tubes to give the concentrations indicated below. 1 μL of buffer was added to the remaining tube, which served as a control. The mixtures were incubated at 30°C. After 1 hour, samples were taken, diluted, mixed with host
20 cells and overlaid onto solid medium. After overnight incubation at 37°C, plaques were counted. The results are summarized below in TABLE XXIV.

TABLE XXIV

	Inhibitor	Inhibitor Concentration	PFU's
5	Hydroxymethylglutamate	0.25%	60×10^4
	Hydroxymethylmethionine	0.25%	68×10^5
	Hydroxymethylserine	0.25%	25×10^3
	Hydroxymethylglutamate	0.1%	12×10^6
	Hydroxymethylmethionine	0.1%	28×10^6
	Hydroxymethylserine	0.1%	27×10^5
10	Hydroxymethylglutamate	0.05%	9×10^7
	Hydroxymethylmethionine	0.05%	7×10^6
	Hydroxymethylserine	0.05%	7×10^6
	None	-	9×10^8

EXAMPLE 25

89 μ L of blood plasma and 10 μ L of T4 stock
 15 solution were added to each of ten tubes. 1 μ L of an
 appropriate hydroxymethylserine stock solution was
 added to each of three tubes to give the
 concentrations indicated below. 1 μ L of an
 appropriate hydroxymethyl- β -alanine stock solution
 20 was added to each of another three tubes to give the
 concentrations indicated below. 1 μ L of an
 appropriate hydroxymethylglycine stock solution was
 added to each of another three tubes to give the
 concentrations indicated below. 1 μ L of buffer was
 25 added to the remaining tube, which served as a
 control. The mixtures were incubated at 30°C. After
 1 hour, samples were taken, diluted, mixed with host
 cells and overlaid onto solid medium. After
 overnight incubation at 37°C, plaques were counted.
 30 The results are summarized below in TABLE XXV.

TABLE XXV

Inhibitor	Inhibitor Concentration	PFU's
Hydroxymethylserine	0.25%	8×10^4
Hydroxymethyl- β -alanine	0.25%	15×10^4
Hydroxymethylglycine	0.25%	9×10^3
Hydroxymethylserine	0.1%	14×10^5
Hydroxymethyl- β -alanine	0.1%	33×10^5
Hydroxymethylglycine	0.1%	8×10^4
Hydroxymethylserine	0.05%	21×10^6
Hydroxymethyl- β -alanine	0.05%	36×10^6
Hydroxymethylglycine	0.05%	26×10^5
None	-	12×10^8

The following is a listing of virucidal activities observed by the present inventors for a variety of hydroxymethyl derivatives (virucidal activity being expressed in terms of PFU's):

$<10^3$

Hydroxymethylglycine

Hydroxymethylphosphonomethylglycine

Hydroxymethyl-p-aminohippurate

Hydroxymethylpropargylglycine

Hydroxymethyl-o-phosphoserine

Hydroxymethylaminoethyl-phosphonic acid

Hydroxymethyllleucine

Hydroxymethyl- β -alanine

Hydroxymethylcysteine

Hydroxymethylphenylalanine

-45-

10³

Hydroxymethylaminophenylacetic acid
Hydroxymethyl-o-phosphorylethanolamine
Hydroxymethylalanine
Hydroxymethylserine
Hydroxymethylvaline
Hydroxymethylmethionine
Hydroxymethylglutamate

5

10⁴

10

Hydroxymethylaspartate
Hydroxymethyllysine
Hydroxymethylproline

10⁵

15

Hydroxymethylmercaptopropionylglycine
Hydroxymethylmercaptoethylamine
Hydroxymethylaminoethyl hydrogen sulfate
Hydroxymethylaminoethanol
Hydroxymethylpenicillamide
Hydroxymethylhydantion
Hydroxymethylornithine
Hydroxymethylthreonine

20

10⁶

Hydroxymethylcysteine
Hydroxymethylaminopropanol

-46-

10⁷

Hydroxymethyluridine

Hydroxymethylphthalimide

Dimethylurea

5 Hydroxymethylcysteineethyl ester

Hydroxymethyllleucinamide

Hydroxymethylarginine

Hydroxymethyltyrosine

10⁸

10 Hydroxymethyldeoxyuridine

4-hydroxymethylimidazole

6-hydroxymethylpterin

Hydroxymethylacrylamide

Hydroxymethylcytosine

15 Hydroxymethyl-6-methyluracil

Hydroxymethylnicotinamide

Hydroxymethyl-trp-gly-gly

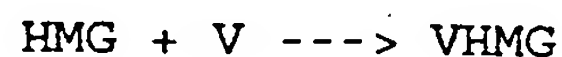
Hydroxymethylglutamine

Diazolidinylurea

20 Imidazolidinylurea

Kinetic Studies

A kinetic model was developed to monitor the complexation between hydroxymethylglycinate (HMG) and a virus. In the kinetic model it was assumed that
5 the reaction of one molecule of HMG with a virus was sufficient to inactivate the virus. Therefore, the overall reaction can be expressed by the simple rate law:



10 where "V" is a virus. A large excess of HMG was present in the reaction conditions and therefore a pseudo first order equation could be used. Furthermore, it was assumed that in a blood bag, no
15 mass transfer effects exist which would affect the reaction kinetics.

It was found that the rate of inactivation follows the model prediction and that the virus can be inactivated in plasma, whole blood and red blood cell concentrate (RBC). The rate is also increased
20 as the temperature of the reaction conditions is increased.

Inactivation of SV4-0, Reovirus, Porcine Parvo virus and Polio virus were demonstrated. For SV4-0, Reovirus and Polio virus, the number of viable cells
25 was reduced below detectable limits. Plasma hemoglobin, potassium, sodium, 2,3-DPG, ATP and lactate were unchanged with reference to a control when treated with HMG at a final concentration of 1600 ppm.

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The embodiments of the present invention recited herein are intended to be merely exemplary and those skilled in the art will be able to make numerous variations and modifications to it without departing
5 from the spirit of the present invention. All such variations and modifications are intended to be within the scope of the present invention as defined by the claims appended hereto.

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CLAIMS

WHAT IS CLAIMED IS:

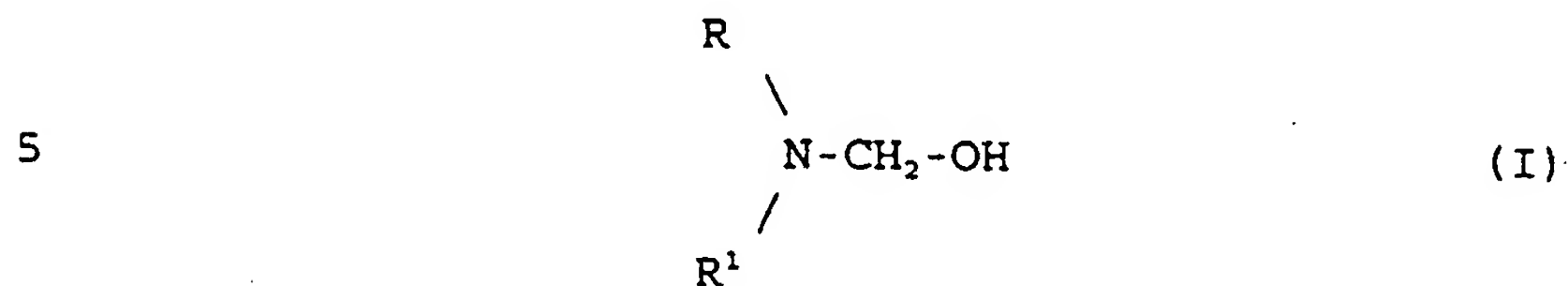
1. A method of inactivating a microorganism in a biological fluid, comprising contacting said biological fluid with an effective amount of a microorganism-inactivating hydroxymethylamine (HMA).

2. The method of claim 1 wherein said microorganism is selected from the group consisting of bacteria, viruses, yeasts and molds.

3. The method of claim 2, wherein said microorganism is a bacterium.

4. The method of claim 2, wherein said microorganism is a virus.

5. The method of claim 1, wherein said HMA is a compound of Formula (I)



wherein:

10 R is chosen from the group consisting of hydrogen, alkyl, aryl, substituted alkyl, substituted aryl;

15 R¹ is chosen from the group consisting of acid-, amide-, hydroxy- or mercapto-functional alkyl; acid-, amide-, hydroxy- or mercapto-functional aryl; acid-, amide-, hydroxy- or mercapto-functional substituted alkyl; and acid-, amide-, hydroxy- or mercapto-functional substituted aryl;

20 or R and R¹ may be joined together to form an acid, amide or hydroxy-functional heterocyclic structure.

6. The method of claim 5, wherein said acid functional group is selected from the group consisting of carboxylate, phosphate, phosphonate, sulfate and sulfonate.

7. The method of claim 6, wherein said acid functional group is a carboxylate.

8. The method of claim 5, wherein said hydroxymethylamine is selected from the group consisting of hydroxymethylglycinamide, hydroxymethylpenicillinamide, hydroxymethylleucinamide, 5 hydroxymethylacrylamide and hydroxymethylnicotinamide.

9. The method of claim 5, wherein said hydroxymethylamine is selected from the group consisting of hydroxymethylglycine, hydroxymethylphosphonomethylglycine, hydroxymethyl-p-aminohippuric 5 acid, hydroxymethylpropargylglycine, hydroxymethyl-o-phosphothreonine, hydroxymethylaminoadipic acid, hydroxymethyl-o-phosphoserine, hydroxymethylamino-

-51-

ethylphosphonic acid, hydroxymethyllleucine,
hydroxymethyl- β -alanine, hydroxymethylcysteine,
10 hydroxymethylfolic acid, hydroxymethylaminophosphono-
butyric acid, hydroxymethylphenylalanine,
hydroxymethylaminophenylacetic acid, hydroxymethyl-o-
phosphorylethanolamine, hydroxymethylalanine,
hydroxymethylserine, hydroxymethylvaline,
15 hydroxymethylmethionine, hydroxymethylglutamic acid,
hydroxymethylaspartic acid, hydroxymethyllysine,
hydroxymethylproline, hydroxymethylmercaptopropionyl-
glycine, hydroxymethylmercaptoethylamine,
hydroxymethylaminoethyl hydrogen sulfate,
20 hydroxymethylaminoethanol, hydroxymethyl-
penicillamine, hydroxymethylhydantoin,
hydroxymethylornithine, hydroxymethylcysteine,
hydroxymethylaminopropanol, hydroxymethyldiethanol-
amine and salts thereof.

10. The method of claim 9, wherein said
hydroxymethylamine is hydroxymethylglycine or a salt
thereof.

11. The method of claim 1, wherein said
hydroxymethylamine and biological fluid are combined
to produce a final concentration of
hydroxymethylamine in said biological fluid of
5 approximately 0.05 % - 3.0 % by weight.

12. The method of claim 1, wherein said
biological fluid and hydroxymethylamine are contacted
for a period of time from 0.5 hours to 4 hours.

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13. The method of claim 1, wherein said biological fluid is contacted with a hydroxymethylamine at a temperature of between about 4° C and about 30° C.

14. The method of claim 1, wherein said biological fluid is whole blood or blood components.

15. The method of claim 14, wherein said blood components are selected from the group consisting of red blood cells, red blood cell concentrate, platelets, platelet concentrate, platelet rich
5 plasma, platelet poor plasma, source plasma (plasmaphoresis plasma), fresh frozen plasma and plasma proteins.

16. The method of claim 1, wherein said biological fluid is selected from the group consisting of lymph, cerebrospinal fluid, semen and saliva.

17. A method of processing a biological fluid intended for administration to an individual in need thereof, said method comprising the steps of:

- 5 (a) treating the biological fluid with an effective amount of a pathogen-inactivating hydroxymethylamine, thereby producing a treated biological fluid; and
- (b) after said treating step, removing free
10 hydroxymethylamine from the treated biological fluid.

18. A method of treating an individual in need of a biological fluid, said method comprising the steps of:

- 5 (a) treating the biological fluid with an effective amount of a pathogen-inactivating hydroxymethylamine, thereby producing a treated biological fluid; and
- (b) administering the treated biological fluid to the individual in need thereof.

19. A method of treating a biological fluid, said method comprising combining an effective amount of a virus-inactivating hydroxymethylamine with said biological fluid, whereby at least about a 10-fold
5 reduction in plaque forming units of virus is realized.

INTERNATIONAL SEARCH REPORT

International Application No.
US 96/11152

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/16 A61K31/195 A61K31/13 A61K31/40 A61K31/415
A61K31/455 A61K31/505 A61K31/66

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,3 981 772 (POVERENNY ET AL.) 21 September 1976	1,2,4-7, 9-13,17, 19
Y	see the whole document	14,15
Y	US,A,4 833 165 (LOUDERBACK) 23 May 1989 see abstract see column 1, line 55 - line 66	14,15
Y	JOURNAL OF PHARMACY AND PHARMACOLOGY, vol. 35, no. 11, 1983, pages 712-717, XP002017821 M.J. GIDLEY ET AL.: "MECHANISMS OF ANTIBACTERIAL FORMALDEHYDE DELIVERY FROM NOXYTHIOLIN AND OTHER "MASKED-FORMALDEHYDE" COMPOUNDS" see page 716, right-hand column, paragraph 3 - page 717, paragraph 2	14,15
	-/--	

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search

6 November 1996

Date of mailing of the international search report

28. 11. 96

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 96/11152

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,81 00188 (SUTTON LABORATORIES) 5 February 1981 cited in the application see abstract; claims; examples 1-5,10 ---	1-3,5-7, 9-13, 17-19
X	BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE, vol. 87, no. 5, 1979, pages 489-491, XP002017822 E.V. MITSEVICH ET AL.: "EFFECT OF FORMALDEHYDE AND ITS AMINOMETHYLOL DERIVATIVES ON STRAINS OF ESCHERICHIA COLI WITH VARIOUS DEFECTS OF DNA REPAIR SYSTEMS" see the whole document ---	1-3,5-7, 9-13,19
X	JOURNAL OF PHARMACY & PHARMACOLOGY, vol. 42, no. 8, 1990, pages 589-590, XP002017823 J.I. BLENKARHN ET AL.: "IN-VITRO ANTIBACTERIAL ACTIVITY OF NOXYTHIOLIN AND TAUROLIDINE" see the whole document ---	1-3,11, 14,15,19
X	REVUE DE L'INSTITUT PASTEUR DE LYON, vol. 13, no. 2, 1980, pages 209-215, XP002017824 G. GARRIGUE ET AL.: "STUDY OF THE IN VITRO VIRUCIDAL ACTIVITY OF NOXYTIOLIN" see the whole document ---	1,2,4, 11,12,19
X	BRITISH JOURNAL OF UROLOGY, vol. 62, no. 4, 1988, pages 306-310, XP002017825 A.C. BUCK: "THE USE OF NOXYTHIOLIN AS AN ANTISEPTIC IRRIGANT IN UPPER URINARY TRACT DRAINAGE FOLLOWING PERCUTANEOUS NEPHROLITHOTOMY" see the whole document ---	1-3,11, 19
X	DE,A,21 35 542 (BOEHRINGER MANNHEIM) 25 January 1973 see the whole document ---	1,2,4,5, 14,19
X	US,A,3 239 415 (G.E. UNDERWOOD ET AL.) 8 March 1966 see the whole document ---	1,2,4, 14,19

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INTERNATIONAL SEARCH REPORT

International Application No
US 96/11152

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DATABASE WPI Section Ch, Week 8740 Derwent Publications Ltd., London, GB; Class A60, AN 87-280956 XP002017826 & JP,A,62 195 304 (DAINIPPON INK CHEM KK) , 28 August 1987 cited in the application see abstract -----</p>	1-19

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/ 11152

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 1-16, 18, 19
is(are) directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
Claims searched completely: 8-10
Claims searched incompletely: 1-7, 11-19
Please see next page.
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

In view of the large number of compounds which are theoretically contained within the definition of claim 1 and defined by the general formula of claim 5, the search was limited to the inventive part of the molecule and to the compounds mentioned in the claims 8-10 (PCT: Art. 6; Guidelines. Part B, Chapt II 7. last sentence and Chapt III, 3-7).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/11152

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-3981772	21-09-76	DE-A- 2528584	11-03-76
		FR-A- 2282906	26-03-76
		JP-A- 52012921	31-01-77
US-A-4833165	23-05-89	NONE	
WO-A-8100188	05-02-81	US-A- 4337269	29-06-82
		AT-T- 9534	15-10-84
		CA-A- 1156150	01-11-83
		EP-A- 0032500	29-07-81
DE-A-2135542	25-01-73	NONE	
US-A-3239415	08-03-66	GB-A- 993601	